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(54) Title: COMPOSITIONS COMPRISING CONJUGATES OF STABLE, ACTIVE, HUMAN OB PROTEIN WITH ANTIBODY FC CHAIN AND METHODS (57) Abstract The present invention relates to OB protein compositions and related methods. Provided herein are OB protein suspensions which are stable and active at physiologic pH. Such OB protein suspensions are useful for the treatment or modulation of weight adiposity level, diabetes, and other conditions.			

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COMPOSITIONS COMPRISING CONJUGATES OF STABLE, ACTIVE, HUMAN OB PROTEIN
WITH ANTIBODY FC CHAIN AND METHODS

Field of the Invention

5 The present invention relates to stable,
active human OB protein compositions at high
concentrations and at or near physiologic pH. Also
provided are related compositions, methods of
manufacture and methods of using such compositions.

10

Background

 Although the molecular basis for obesity is
largely unknown, the identification of the "OB gene"
and protein encoded ("OB protein") has shed some light
15 on mechanisms the body uses to regulate body fat
deposition. Zhang et al., Nature 372: 425-432 (1994);
see also, the Correction at Nature 374: 479 (1995).
PCT publication No. WO 96/05309, published February 22,
1996, entitled, "Modulators of Body Weight,
20 Corresponding Nucleic Acids and Proteins, and
Diagnostic and Therapeutic Uses Thereof" fully sets
forth OB protein and related compositions and methods,
and is herein incorporated by reference. An amino acid
sequence for human OB protein is set forth at
25 WO 96/05309 (herein incorporated by reference) SEQ ID
NOS. 4 and 6 (at pages 172 and 174 of that
publication), and the first amino acid residue of the
mature protein is at position 22 and is a valine
residue. The mature protein is 146 residues (or 145 if
30 the glutamine at position 49 is absent, SEQ ID NO. 6).

 The OB protein is active in vivo in both
ob/ob mutant mice (mice obese due to a defect in the
production of the OB gene product) as well as in
normal, wild type mice. The biological activity
35 manifests itself in, among other things, weight loss.
See generally, Barinaga, "Obese" Protein Slims Mice,

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Science 269: 475-476 (1995) and Friedman, "The Alphabet of Weight Control," Nature 385: 119-120 (1997). It is known, for instance, that in *ob/ob* mutant mice, administration of OB protein results in a decrease in serum insulin levels, and serum glucose levels. It is also known that administration of OB protein results in a decrease in body fat. This was observed in both *ob/ob* mutant mice, as well as non-obese normal mice. Pellemounter et al., Science 269: 540-543 (1995); Halaas et al., Science 269: 543-546 (1995). See also, Campfield et al., Science 269: 546-549 (1995) (Peripheral and central administration of microgram doses of OB protein reduced food intake and body weight of *ob/ob* and diet-induced obese mice but not in *db/db* obese mice.) In none of these reports have toxicities been observed, even at the highest doses.

For preparation of a pharmaceutical composition for injection in humans, it has been observed that the human amino acid sequence is insoluble at physiologic pH at relatively high concentrations, such as above about 2 mg active protein/milliliter of liquid. Dosages in the milligram protein per kilogram body weight range, such as .5 or 1.0 mg/kg/day or below, are desirable for injection of therapeutically effective amounts into larger mammals, such as humans. An increase in protein concentration is necessary to avoid injection of large volumes, which can be uncomfortable or possibly painful to the patient.

With the advances in recombinant DNA technology, the availability of recombinant proteins for therapeutic use has engendered advances in protein formulation. A review article describing protein

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modification and fusion proteins is Francis, *Focus on Growth Factors* 3:4-10 (1992).

One such modification is the use of the Fc region of immunoglobulins. Antibodies comprise two functionally independent parts, a variable domain known as "Fab", which binds antigen, and a constant domain, known as "Fc" which provides the link to effector functions such as complement or phagocytic cells. The Fc portion of an immunoglobulin has a long plasma half-life, whereas the Fab is short-lived. Capon et al., *Nature* 337: 525-531 (1989).

Therapeutic proteins have been constructed using the Fc domain to provide longer half-life or to incorporate functions such as Fc receptor binding, protein A binding, complement fixation and placental transfer which all reside in the Fc proteins of immunoglobulins. *Id.* For example, the Fc region of an IgG1 antibody has been fused to the N-terminal end of CD30-L, a molecule which binds CD30 receptors expressed on Hodgkin's Disease tumor cells, anaplastic lymphoma cells, T-cell leukemia cells and other malignant cell types. *See*, U.S. Patent No. 5,480,981. IL-10, an anti-inflammatory and anti-rejection agent has been fused to murine Fcγ2a in order to increase the cytokine's short circulating half-life. Zheng, X. et al., *The Journal of Immunology*, 154: 5590-5600 (1995). Studies have also evaluated the use of tumor necrosis factor receptor linked with the Fc protein of human IgG1 to treat patients with septic shock. Fisher, C. et al., *N. Engl. J. Med.*, 334: 1697-1702 (1996); Van Zee, K. et al., *The Journal of Immunology*, 156: 2221-2230 (1996). Fc has also been fused with CD4 receptor to produce a therapeutic protein for treatment of AIDS. *See*, Capon et al., *Nature*, 337:525-531 (1989). In addition, the

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N-terminus of interleukin 2 has also been fused to the Fc portion of IgG1 or IgG3 to overcome the short half life of interleukin 2 and its systemic toxicity. See, Harvill et al., Immunotechnology, 1: 95-105 (1995).

5 For insulin, suspension preparations have been reported. But, those conditions which are applicable to insulin are not predictive of those conditions which may be applicable to any other protein, including OB protein. Insulin is a fairly
10 small protein, having particular physical and chemical characteristics; these characteristics are important to determining conditions for formulation. Brange, Galenics of Insulin, Springer-Verlag 1987 describes insulin suspensions (p.36); see also, Schlichtkrull et
15 al. Insulin Preparations with Prolonged Effect, pp. 729-777 In: Hassellblatt et al., Handbook of Experimental Pharmacology New Series, Vol. XXXII-1/2 Springer-Verlag Berlin, Heidelberg, New York (1975).

To date, there have been no reports of stable
20 preparations of human OB protein at concentrations of at least about 2 mg/ml at physiologic pH, and further, no reports of stable concentrations of active human OB protein at least about 50 mg/ml or above. Moreover, a frozen or lyophilized form may be used to improve
25 shelf-stability but, are less desirable than the ready-to-use suspension forms described here. A frozen form requires storage at a constant frozen temperature which may not be possible due to defrost cycles of consumer-grade refrigerators and freezers. Furthermore, a
30 lyophilized form must be diluted and mixed which is inconvenient and may compromise patient compliance. From a producer's point of view, manufacturing, storing and shipping of frozen liquid is expensive and requires far more supervision than distribution of a ready to

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use formulation. Also, manufacture or otherwise making available a suitable diluent for a lyophilized formulation is more costly and less efficient than not requiring such diluent. There exists a need for concentrated forms of human pharmaceutical compositions containing active OB protein which can be delivered by injection at low volumes. The present invention fulfills these requirements.

10 Summary of the Invention

The present invention stems from the observation that certain suspension formulations allow the preparation of stable OB protein in concentrations of at least about 2 mg/ml at physiological pH. As used herein, the term, "physiological pH" refers to a pH of about 6.0 to about 8.0. Use of such compositions allow for delivery of relatively low volumes of OB protein therapeutic. As further disclosed herein, the use of precipitating agents allows for the preparation of suspensions of human OB protein having the characteristics of:

1. Improved stability at physiological pH as compared to the same human OB protein in solution form. Demonstrated below in the working examples are human OB protein suspensions at concentrations of 10 mg/ml or higher and at pH 7, which, when compared to the same concentration in solution at the highest pH which allows solubility (pH 4 for human OB protein in its native form, which is not even physiologic pH) have better HPLC (high pressure liquid chromatography) profiles. Also demonstrated below is a Fc-OB fusion protein suspension at a concentration of 5 mg/ml at about pH 7 which shows less degradation products upon

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storage than a comparable Fc-OB fusion protein solution.

2. Sustained injection time-release profile as compared to the same human OB protein in solution form. The working examples below demonstrate a
5 sustained-release effect using the present highly concentrated human OB protein suspensions. Such sustained release effect is advantageous in that the activity of material is maintained over a relatively
10 longer period of time, and thus there is a relatively higher potency, and the need for fewer injections as compared with human OB protein in solution.

Thus, one object of the present invention is a stable preparation of active human OB protein at
15 physiologic pH, having a concentration of at least about 2 mg protein/ml. For instance, a stable preparation of active human OB protein at a concentration of at least 2.0 mg/ml and a pH of 7.0 is provided. The upper limit of concentration is that
20 suspension form which is available for administration to a human, and, as described below, working examples have provided a concentration of as high as 100 mg/ml at physiologic pH (e.g., between pH 6.0 and pH 8.0).

In another aspect, the present invention
25 relates to a stable preparation of active human OB protein within the pH range of about 6.0 to about 8.0 having a concentration of at least about 10 mg/ml.

In still another aspect, the present invention relates to a stable preparation of active
30 human OB protein derivatized by the attachment of an Fc region of an immunoglobulin at a concentration of at least about 0.5 mg/ml and a pH of about 6.0 to about 8.0. More particularly, stable preparations of active

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Fc-OB fusion protein at a concentration of 5 mg/ml to 50 mg/ml at about pH 7.5 are provided.

In yet other aspects, the present invention provides formulations for stable, active human OB protein at a concentration of 2 mg protein/ml or above, at pH 6.5 to pH 7.5. More particularly, formulations for stable, active human OB protein at a concentration of 20 mg/ml to 100 mg/ml at pH 7.0 are provided.

In another aspect, the present invention provides formulations for stable, active human OB protein at a concentration of 10 mg/ml or above at a pH of 5.0 to 8.0.

In still another aspect, the present invention provides formulations for stable, active human OB protein derivatized by the attachment of an Fc region of an immunoglobulin at a concentration of 0.5 mg/ml or above and a pH of about 6.0 to about 8.0. More particularly, formulations for stable, active Fc-OB fusion protein at a concentration of 5 mg/ml to 50 mg/ml at about pH 7.5 are provided.

Yet other aspects of the present invention include pharmaceutical compositions of the above, methods for manufacturing such compositions, and methods of treatment using present compositions, and methods of manufacturing medicaments containing the present compositions for such treatment.

Brief Description of the Drawings

FIGURES 1A and 1B are dose response curves in mice for (1A) the present human OB suspension of Example 1 and (1B) a control human OB protein solution as described in Example 1.

FIGURE 2 is a graph illustrating OB serum levels in dogs for the present OB protein suspension

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and for a control human OB protein solution as described in Example 1.

FIGURE 3 is a reversed phase high pressure liquid chromatography (RP-HPLC) tracing of human OB protein formulations at 37°C for seven weeks: the human OB protein zinc suspension of Example 1 is the middle tracing, the human OB protein solution control of Example 1 is the top line and a human OB protein suspension kept at -80°C for 56 days is the bottom line.

FIGURE 4 is a RP-HPLC tracing of human OB protein formulations at 19°C for 56 days: human OB solution of Example 1 is the top line, the human OB crystalline suspension of Example 2 is the middle line, and the human OB crystalline suspension of Example 2 at -80°C for 56 days is the bottom line.

FIGURES 5A-5C are the DNA sequence (SEQ ID NO: 1) and amino acid sequence (SEQ ID NO: 2) of a human metFc-OB protein.

FIGURES 6A-6C are the DNA sequence (SEQ ID NO: 3) and amino acid sequence (SEQ ID NO: 4) of a human metFc-OB protein variant.

FIGURE 7 is a graph depicting the rate of formation of iso asp at asp 108 (asp 335 using the numbering according to SEQ ID NO: 4), as determined by reverse phase HPLC, for recombinant methionyl human Fc-OB protein.

Detailed Description

The present stable, active OB protein compositions are generally classified as suspensions, in that the protein moiety is precipitated and suspended in a liquid moiety. The compositions contain an active OB protein moiety, a precipitating agent, a

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pH modulating agent, and a liquid carrier. The present OB proteins are either amorphous or in crystalline form.

Preferably, for use as a therapeutic or cosmetic composition in humans, OB protein with the amino acid sequence of native human OB protein (see Zhang et al., Nature, supra), optionally with an N-terminal methionyl residue incident to bacterial expression, is used. See, PCT Publication WO 96/05309, herein incorporated by reference, for recombinant DNA means to prepare the present OB proteins which may be used. One may make changes in selected amino acids so long as such changes preserve the overall folding or activity of the protein. Table 1, below sets forth conserved amino acid substitutions which may be used, in terms of particular characteristics (basic, acidic, polar, hydrophobic, aromatic, and size (small)). See, in general, Ford et al., Protein Expression and Purification 2: 95-107, 1991, which is herein incorporated by reference. Small amino terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification, such as a poly-histidine tract, an antigenic epitope or a binding domain, may also be present.

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Table 1

Conservative Amino Acid Substitutions

Basic:	arginine lysine histidine
Acidic:	glutamic acid aspartic acid
Polar:	glutamine asparagine
Hydrophobic:	leucine isoleucine valine
Aromatic:	phenylalanine tryptophan tyrosine
Small:	glycine alanine serine threonine methionine

5 Generally, human OB proteins which will display increased stability in the present suspensions will be those which, upon exposure to physiologic pH, have an exposed hydrophobic region when in solution. Additionally, human OB proteins derivatized by the
10 attachment of an Fc region of an immunoglobulin to the OB protein moiety display increased stability in the present suspensions.

 Generally, an Fc region of an immunoglobulin may be genetically or chemically fused to a human OB
15 protein. Preferably, the Fc region is fused at the N-terminus of the OB protein. See copending U.S. application Serial No. 08/770,973, filed December 20, 1996, herein incorporated by reference, for preferred Fc-OB fusion proteins.

20 Preferably, an Fc region with the amino acid sequence of the human immunoglobulin IgG-1 heavy chain (see Ellison, J.W. et al., Nucleic Acids Res. 10: 4071-

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4079 (1982)) is used. A preferred Fc region is set forth in SEQ ID NO: 2 (see Figure 5). The recombinant Fc-OB sequence of SEQ ID NO: 2 is a 378 amino acid Fc-OB protein (not counting the methionine residue). The first amino acid residue of the Fc-OB protein in Figure 5, glutamic acid, is referred to as +1 with the methionine at the -1 position. Variants or analogs of the Fc portion may be constructed by, for example, making various substitutions of amino acid residues or base pairs.

Cysteine residues can be deleted or replaced with other amino acids to prevent formation of disulfide crosslinks of the Fc sequences. In particular, amino acid at position 5 of SEQ ID NO: 2 is a cysteine residue. One may remove the cysteine residue at position 5 or substitute it with one or more amino acids. For example, an alanine residue may be substituted for the cysteine residue at position 5 resulting in a variant amino acid sequence. Likewise, the cysteine at position 5 of SEQ ID NO: 2 may be substituted with a serine or other amino acid residue or deleted.

A variant or analog may also be prepared by deletion of the amino acids at positions 1, 2, 3, 4 and 5, resulting in a 373 amino acid Fc-OB protein (not counting the methionine residue). This sequence is set forth in SEQ ID NO:4 (see Figure 6). Substitutions at these positions can also be made and are within the scope of this invention.

Modifications may also be made to introduce four amino acid substitutions to ablate the Fc receptor binding site and the complement (C1q) binding site. According to the numbering of SEQ ID NO: 4, these variant modifications include leucine at position 15

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substituted with glutamic acid, glutamic acid at position 98 substituted with alanine, and lysines at positions 100 and 102 substituted with alanines.

Likewise, one or more tyrosine residues can be replaced by phenylalanine residues as well. As described above, one may make changes in selected amino acids as long as such changes preserve the overall folding or activity of the fusion protein.

Furthermore, the Fc region may be also linked to the human OB protein of the Fc-OB fusion protein by "linker" moieties whether chemical or amino acids of varying lengths. Such chemical linkers are well known in the art. Amino acid linker sequences can include but are not limited to:

- (a) ala, ala, ala;
- (b) ala, ala, ala, ala;
- (c) ala, ala, ala, ala, ala;
- (d) gly, gly;
- (e) gly, gly, gly;
- (f) gly, gly, gly, gly, gly;
- (g) gly, gly, gly, gly, gly, gly, gly, gly;
- (h) gly-pro-gly;
- (i) gly, gly, pro, gly, gly; and
- (j) any combination of subparts (a) through (i).

The present precipitating agents may be a salt having a cationic component, and may be selected from among calcium, magnesium, zinc, sodium, iron, cobalt, manganese, potassium and nickel. Preferably, the salt will be compatible for use in a pharmaceutical composition.

Alternatively precipitating agents may be selected from among agents which are pharmaceutically acceptable yet known to precipitate protein, such as

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polyethylene glycols, or other water soluble polymers as set forth in the next paragraphs. A useful precipitating agent will induce precipitation of OB protein at neutral pH but is reversible or
5 redissolveable upon dilution with physiologically compatible solvents. Without an appropriate precipitating agent, OB protein precipitates at neutral pH to a form that is not reversible by dilution with physiologically compatible solvents.

10 The pH range is preferably from about pH 4.0 to about pH 8.0, and more preferably from about 6.5 to about 7.5. The most preferable pH for a pharmaceutical composition is that in which the OB protein used may retain its maximum biological activity at the selected
15 protein concentration. At non-physiologic pH, the present OB protein suspensions may also have advantages. At pH below 5.0, the present OB protein suspensions may be more stable (in terms of shelf-life) than equal concentrations of OB protein in solution at
20 equal pH. For example at pH 4.0, and a concentration of 50 mg/ml, the present suspensions may have greater biological activity upon in vivo administration than would the equivalent solution.

The buffer may be selected from among those
25 which attain the desired pH while not altering the precipitating characteristics of the composition. Preferably, buffers will be acceptable for a pharmaceutical formulation. Tris, MES, and PIPES are acceptable for both the amorphous and crystalline
30 forms. Phosphate is a preferred buffer for the crystalline forms.

The final suspension will preferably have a concentration of 5 mg/ml to 100 mg/ml for ease of therapeutic administration.

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Methods of Use

Therapeutic. Therapeutic uses include weight modulation, the treatment or prevention of diabetes, blood lipid reduction (and treatment of related conditions), increasing lean body mass and increasing insulin sensitivity. In addition, the present compositions may be used for manufacture of one or more medicaments for treatment or amelioration of the above conditions. Methods of administration will typically be by injection, although other means, such as pulmonary delivery may be used. See PCT WO 96/05309, incorporated by reference at page 83 et seq., for example. The present suspensions may be spray-dried into particles having an average size of less than 10 microns, or more preferably, 0.5 to 5 microns.

Weight Modulation. The present compositions and methods may be used for weight reduction. Viewed another way, the present compositions may be used for maintenance of a desired weight or level of adiposity. As has been demonstrated in murine models (see supra), administration of the present OB protein results in weight loss. The body mass lost is primarily of adipose tissue, or fat. Such weight loss can be associated with the treatment of concomitant conditions, such as those below, and therefore constitute a therapeutic application. In addition, cosmetic uses are provided herein if weight modulation is solely for improvement in appearance.

Treatment of Diabetes. The present compositions and methods may be used in the prevention or treatment of Type II diabetes. As Type II diabetes can be correlated with obesity, use of the present invention to reduce weight (or maintain a desired

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weight, or reduce or maintain an adiposity level) can also alleviate or prevent the development of diabetes. Moreover, even in the absence of dosages sufficient to result in weight loss, the present compositions may be
5 used to prevent or ameliorate diabetes.

Blood Lipid Modulation. The present compositions and methods may be used in the modulation of blood lipid levels. Ideally, in situations where solely reduction in blood lipid levels is desired, or
10 where maintenance of blood lipid levels is desired, the dosage will be insufficient to result in weight loss. Thus, during an initial course of therapy of an obese patient, dosages may be administered whereby weight loss and concomitant blood lipid level lowering is
15 achieved. Once sufficient weight loss is achieved, a dosage sufficient to prevent re-gaining weight, yet sufficient to maintain desired blood lipid levels, or other conditions as set forth herein, for example, may be administered. These dosages can be determined
20 empirically, as the effects of OB protein are reversible. E.g., Campfield et al., Science 269: 546-549 (1995) at 547. Thus, if a dosage resulting in weight loss is observed when weight loss is not desired, one would administer a lower dose in order to
25 achieve the desired blood lipid levels, yet maintain the desired weight. See, e.g., PCT Publication WO 97/06816 herein incorporated by reference.

Increasing Lean Mass or Insulin Sensitivity. Ideally, in situations where solely an increase in lean
30 body mass is desired, the dosage will be insufficient to result in weight loss. Thus, during an initial course of therapy of an obese person, dosages may be administered whereby weight loss and concomitant fat tissue decrease/lean mass increase is achieved. Once

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sufficient weight loss is achieved, a dosage sufficient to prevent regaining weight, yet sufficient to maintain desired lean mass increase (or prevention of lean mass depletion) may be administered. For increasing an individual's sensitivity to insulin, similar dosage considerations may be taken into account. Lean mass increase without weight loss may be achieved sufficient to decrease the amount of insulin (or, potentially, amylin, amylin antagonists or agonists, or thiazolidinediones, or other potential diabetes treating drugs) an individual would be administered for the treatment of diabetes. For increasing overall strength, there may be similar dosage considerations. Lean mass increase with concomitant increase in overall strength may be achieved with doses insufficient to result in weight loss. Other benefits, such as an increase in red blood cells (and oxygenation in the blood) and a decrease in bone resorption or osteoporosis may also be achieved in the absence of weight loss. See, e.g., PCT Publication No. WO 97/18833 herein incorporated by reference.

Combination Therapies. The present compositions and methods may be used in conjunction with other therapies, such as altered diet and exercise. Other medicaments, such as those useful for the treatment of diabetes (e.g., insulin and possibly amylin, antagonists or agonists thereof, thiazolidinediones (see, e.g., PCT Publication No. WO 98/08512 herein incorporated by reference), or other potential diabetes treating drugs), cholesterol and blood pressure lowering medicaments (such as those which reduce blood lipid levels or other cardiovascular medicaments), activity increasing medicaments (e.g., amphetamines), diuretics (for liquid elimination), and

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appetite suppressants (such as agents which act on neuropeptide γ receptors or serotonin reuptake inhibitors). Such administration may be simultaneous or may be in seriatim. In addition, the present methods
5 may be used in conjunction with surgical procedures, such as cosmetic surgeries designed to alter the overall appearance of a body (e.g., liposuction or laser surgeries designed to reduce body mass, or implant surgeries designed to increase the appearance
10 of body mass). The health benefits of cardiac surgeries, such as bypass surgeries or other surgeries designed to relieve a deleterious condition caused by blockage of blood vessels by fatty deposits, such as arterial plaque, may be increased with concomitant use
15 of the present compositions and methods. Methods to eliminate gall stones, such as ultrasonic or laser methods, may also be used either prior to, during or after a course of the present therapeutic methods. Furthermore, the present methods may be used as an
20 adjunct to surgeries or therapies for broken bones, damaged muscle, or other therapies which would be improved by an increase in lean tissue mass.

The following examples are offered to more fully illustrate the invention, but are not to be
25 construed as limiting the scope thereof. Example 1 sets forth preparation of an amorphous (as opposed to a crystalline, as infra) human OB protein suspension at a concentration of 100 mg/ml at pH 7.0. Example 2 sets forth preparation of a crystalline OB protein
30 suspension. Example 3 demonstrates an improved dose response for OB protein suspensions as compared to OB protein solutions. Example 4 demonstrates the delayed time action profile of the present suspensions in a dog model. Example 5 sets forth preparation of an

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amorphous Fc-OB protein suspension. Examples 6 and 7 demonstrate the improved stability of the present suspensions.

- 5 EXAMPLE 1: Preparation of an amorphous OB protein suspension.

This example illustrates preparation of one of the human OB protein suspensions of the present invention. An amorphous human OB protein suspension
10 was prepared by precipitation with zinc salt. At a final pH of 6.0 to pH 8.0, a concentration of 100 mg protein/ml liquid has been obtained. Also set forth is a control composition, of pH 4.0, for a human OB protein solution.

- 15 Composition:

Protein moiety: recombinant methionyl human OB protein ("rmetHu-leptin") as set forth in SEQ ID NO. 4 of PCT Publication WO 96/05309, beginning with amino acid number 22 (Val) and ending with amino acid
20 number 167, having at its N-terminus a methionyl residue:

Precipitant: Zinc chloride

Buffer: Tris, MES and Pipes

Final pH: 6.0-8.0

- 25 Preparation protocol: Recombinant methionyl human OB protein ("rmetHu-leptin") solution was concentrated to about 40 mg/ml in water for injection, acidified to pH 3.0, with HCl. Zinc chloride was added and the suspension was formed by adjusting the pH to
30 near neutrality (approximately pH 7.0) by adding an appropriate buffer. Tris, MES and PIPES buffer have been successfully used. The final conditions were typically 10 to 15 mM buffer, 20 to 1000 μ M Zinc, pH 6.0 to 8.0 and 10 mg/ml rmetHu-leptin. The

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suspensions have been concentrated by allowing the particles to settle at 4°C for several hours and removing the supernatant. This procedure may be repeated several times until the maximum concentration is obtained, and has been used to obtain about 100 mg/ml suspensions.

Control Composition: Recombinant methionyl human OB protein (as above) solution at 20 mg/ml pH 4.0 containing 10 mM acetate and 5% w/v sorbitol was used as a control composition.

EXAMPLE 2: Preparation of a crystalline OB protein suspension.

This example illustrates the preparation of a crystalline OB protein suspension of the present invention.

Protein moiety: recombinant methionyl human OB protein as in Example 1, above, was used.

Procedure: rmetHu-leptin at a concentration of 15 mg/ml in 1 mM HCl was mixed in a 1:1 ratio with 4 M NaCl, 100 mM Tris, pH 8.5, 2% v/v ethanol, at 4°C. Crystals spontaneously formed by slowly adjusting the temperature over several hours to between 14°C and 25°C and maintaining that temperature for at least 2 hours depending on the duration of warming to the final temperature. The "mother liquor" (i.e., the liquid in which the crystals were grown) was replaced with a more suitable solvent for injection by harvesting the crystals by centrifugation and resuspension in an appropriate crystal stabilizing solvent. A suitable replacement solvent is 20-25% polyethylene glycol (having a molecular weight of about 4000 daltons to about 20,000 daltons, the term "about" meaning that the approximate average of molecular weights for commercial

- 20 -

PEG preparations), appropriate neutral pH buffer, preferably about pH 6.0 to about pH 8.0, and preferably 10 mM phosphate buffer pH 6.0 to pH 7.5, and 2% ethanol v/v. In a typical preparation, some residual salt, usually less than 0.25 M, may remain.

EXAMPLE 3: Improved dose response for OB protein suspensions as compared to OB protein solutions.

This example demonstrates that the present OB protein suspensions are more efficacious than OB protein in solution. Normal lean mice were given daily injections, for 5 days, of either the present suspension, at 1, 10 and 50 mg of protein per kg of body weight, or OB protein solution at the same dose. The mice given the suspension lost more weight per unit of mass of OB protein given than did mice given equal doses of the solution formulation. This is illustrated in FIGURE 1A and FIGURE 1B. FIGURE 1A shows the percent weight change when the suspension of Example 1 was given. FIGURE 1B shows the percent weight change when the control solution of Example 1 was given.

This illustrates that the present suspension at the same dose is more efficacious than that given in solution form. While not wishing to be bound by theory, this may be due to slower absorption rate of the suspension as compared to the solution. The suspension must dissolve before it enters the blood, so this sustained release effect results in higher efficacy. On a mass basis, less protein in suspension needs to be administered than solution. Further, in solution there is no difference between the 10 and 50 mg/kg dose at day 5 (the last day of dosing) (See Table 2, below). The suspension gives a more definitive dose response curve than does the solution formulation.

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TABLE 2
PERCENT WEIGHT CHANGE FROM DAY 0

Day	6
Placebo	1.4
Suspension 1 mg/kg	-1.7
Suspension 10 mg/kg	-5.9
Suspension 50 mg/kg	-9.5
Solution 1 mg/kg	-.7
Solution 10 mg/kg	-3.0
Solution 50 mg/kg	-3.5

5 The data is an average for 5 mice per treatment.

Methods:

Animals: Normal CD-1 mice were used.

Base weight: About 20 grams.

10 Administration: Animals were injected SC each day in the same location for 5 days.

Handling: Animals were group housed, and fed ad libitum.

Compositions:

15 Solution: The rmetHu-leptin solution of Example 1 was used, at a concentration of 20 mg/ml.

Suspension: The rmetHu-leptin suspension of Example 1 was used, at pH 7.0, 10 mM MES buffer, 500 mM Zn, 20 mg/ml.

20 PBS: Phosphate buffered saline was used as a placebo. (Dose responses for controls using suspension liquid or solution liquid alone, with no protein, were similar to PBS, data not shown).

25 EXAMPLE 4: OB protein serum levels in dogs.

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This Example demonstrates the delayed time action profile of the present suspensions.

Methods: Beagle dogs were administered suspension or solution rmetHu-leptin. Serum was drawn, and OB
5 protein levels were measured at the times illustrated in FIGURE 2.

TABLE 3

Time after injection (hours)	Serum concentration Solution	Serum concentration Suspension (crystal)	Serum concentration Suspension (zinc)
0	0	0	0
.5	532	9.4	50.5
1	1047	12.9	130.4
2	1706	51	298.5
4	1480	87	323.5
8	742	298	247.7
12	301	426	241.1
16	109	389	209.6
24	5	90	57.7

10

Compositions used:

Solution: The solution according to Example 1 was used, at a dose of 5 mg/kg/day.

15 Suspension: The suspension according to Example 2 was used. See Table.

Animals: The data presented in Table 3 is for an average of 3 animals. Animals were normal beagle dogs.

20 Handling: Animals were housed individually, and fed ad libitum. Good animal handling practices were obeyed.

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Assay: An antibody assay was used as in Hotta et al, J. Biol. Chem 271: 255327-25331 (1996).

Results: As can be seen from FIGURE 2, the solution concentration peaks at 1 to 2 hours after

5 administration of protein, whereas the suspension concentration peaks much later, after 10-12 hours.

This demonstrates that the suspension maintains a minimum effective dose for a longer time period.

10 EXAMPLE 5: Preparation of an amorphous Fc-OB protein suspension.

This example illustrates preparation of one of the human Fc-OB protein suspensions of the present invention. An amorphous human Fc-OB protein suspension
15 was prepared by precipitation with zinc salt. Also set forth is a control composition, of pH 7.5, for a human Fc-OB protein solution.

Composition:

Protein moiety: recombinant methionyl human
20 Fc-OB protein ("rmetHu-Fc-leptin") as set forth in SEQ ID NO: 4, a 373 amino acid fusion protein, the Fc portion of the protein being amino acids 1-227 and the human OB protein portion of the protein being amino acids 228-373, having at its N-terminus a methionyl
25 residue:

Precipitant: Zinc chloride

Buffer: 100mM Tris

Final pH: 7.5

Preparation protocol: A rmetHu-Fc-leptin
30 solution was concentrated to about 5 mg/ml in 100 mM Tris buffer, pH 7.5. Zinc chloride was added to form the suspension.

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Control Composition: A rmetHu-Fc-leptin solution (as above) at 5 mg/ml, pH 7.5 containing 100 mM Tris was used as a control composition.

- 5 EXAMPLE 6: Stability of the present human OB protein suspensions.

This example demonstrates that both the amorphous and the crystalline forms of the present suspensions are more stable under accelerated stability
10 assay conditions than material in solution.

FIGURE 3 illustrates an RP-HPLC tracing comparing the zinc amorphous form of the present suspension (as in Example 1) with the solution form (also as in Example 1), at 37°C for a period of 7
15 weeks. As can be seen, the present suspension (middle line) has fewer peaks (indicating fewer breakdown products) than the solution form (top line). As a comparison, the bottom line presents the zinc amorphous form which had been stored for 7 weeks at -80°C.

20 FIGURE 4 illustrates an RP-HPLC tracing comparing the crystalline suspension form of Example 2 with the solution form (of Example 1). The materials were stored for 56 days at 19°C. (Storage at 37°C was not a relevant condition, as the crystalline form loses
25 its crystalline structure at 37°C.) FIGURE 4 shows that there are more peaks, indicating more degradation, for the solution form, (main peak = 86%) than for the suspension form (main peak = 94%). The control at the bottom line was the crystalline form stored for 56 days
30 at -80°C. Similar results were observed for 4°C, although degradation occurred more slowly.

The main degradation product appeared to be the aspartate at amino acid position 108 (according to SEQ ID NO: 4 in PCT WO 96/05309, using the "Val"

- 25 -

residue as position number 1). One may empirically select a more stable chemical moiety at this position, such as another amino acid, to improve stability of the molecule. Overall, the suspensions of the present invention are more stable than OB protein in solution.

EXAMPLE 7: Stability of the present human Fc-OB protein suspensions.

This example demonstrates that the amorphous form of the present Fc-OB protein suspensions is more stable under accelerated stability assay conditions than material in solution.

The zinc amorphous form of the present suspension (as in Example 5) and the solution form (also as in Example 5) were put on stability studies by storing both the suspension and solution forms at 4°C, 29°C and 37°C for a period of 2 weeks. As shown in FIGURE 7, the Fc-leptin suspensions stored at 29°C and 37°C show a lower percentage of degradation products related to the aspartate at amino acid position 108 of the OB protein than the Fc-leptin solutions stored at the same temperatures, respectively.

* * *

While the present invention has been described in terms of preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations which come within the scope of the invention as claimed.

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CLAIMS

1. A human OB protein suspension having a pH of about 6.0 to about 8.0 and a concentration of at least 0.5 mg/ml wherein the OB protein is derivatized by the attachment of an Fc region of an immunoglobulin to the N-terminus of the OB protein moiety.

2. A human OB protein suspension of claim 1 wherein the Fc portion of said human Fc-OB protein is selected from the group consisting of:

(a) the Fc amino acid sequences as set forth in SEQ ID NOS: 2 and 4;

(b) the amino acid sequence of subpart (a) having a different amino acid substituted or deleted in one or more of the following positions (using the numbering according to SEQ ID NO: 2):

- (i) one or more cysteine residues replaced by an alanine or serine residue;
- (ii) one or more tyrosine residues replaced by a phenylalanine residue;
- (iii) the amino acid at position 5 replaced with an alanine;
- (iv) the amino acid at position 20 replaced with glutamic acid;
- (v) the amino acid at position 103 replaced with an alanine;
- (vi) the amino acid at position 105 replaced with an alanine;
- (vii) the amino acid at position 107 replaced with an alanine;
- (viii) the amino acids at positions 1, 2, 3, 4, and 5 deleted;

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- (ix) one or more residues replaced
or deleted to ablate the Fc receptor binding site;
(x) one or more residues
substituted or deleted to ablate the complement
5 (C1q) binding site; and
(xi) a combination of subparts i-x;
and
(c) the amino acid sequence of subparts (a)
or (b) having a methionyl residue at the N-
10 terminus.

3. A human OB protein suspension of claim 1
wherein said concentration is at least 5 mg/ml.

15 4. A human OB protein suspension of claim 1
wherein said concentration is between 5 mg/ml and 50
mg/ml.

20 5. A human OB protein suspension of claim 1
wherein said pH is pH 7.0.

6. A human OB protein suspension of claim 2
wherein said human OB protein is rmetHu-Fc-leptin.

25 7. A human OB protein suspension of claim 1
containing a pharmaceutically acceptable precipitating
agent.

30 8. A human OB protein suspension of claim 1
containing a precipitating agent selected from a salt
and a water soluble polymer.

9. A human OB protein suspension of claim 1
containing a precipitating agent selected from among

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calcium, magnesium, zinc, sodium, iron, cobalt,
manganese, potassium and nickel.

10. A method of treating an individual for a
5 condition by administering an effective dose of a human
OB suspension according to claim 1, said condition
selected from among:

- (a) weight modulation,
- (b) adiposity modulation,
- 10 (c) diabetes,
- (d) blood lipid level modulation,
- (e) increase in lean mass, and
- (f) increase in insulin sensitivity.

15 11. A method of preparing a human OB protein
suspension comprising:

- combining, under suitable conditions, a
precipitating agent and a human OB
protein in solution, wherein the OB
20 protein is derivatized by the attachment
of an Fc region of an immunoglobulin to
the N-terminus of the OB protein moiety;
allowing said human OB protein to
precipitate;
- 25 collecting said precipitated human OB
protein; and
optionally, resuspending said human OB
protein in a diluent.

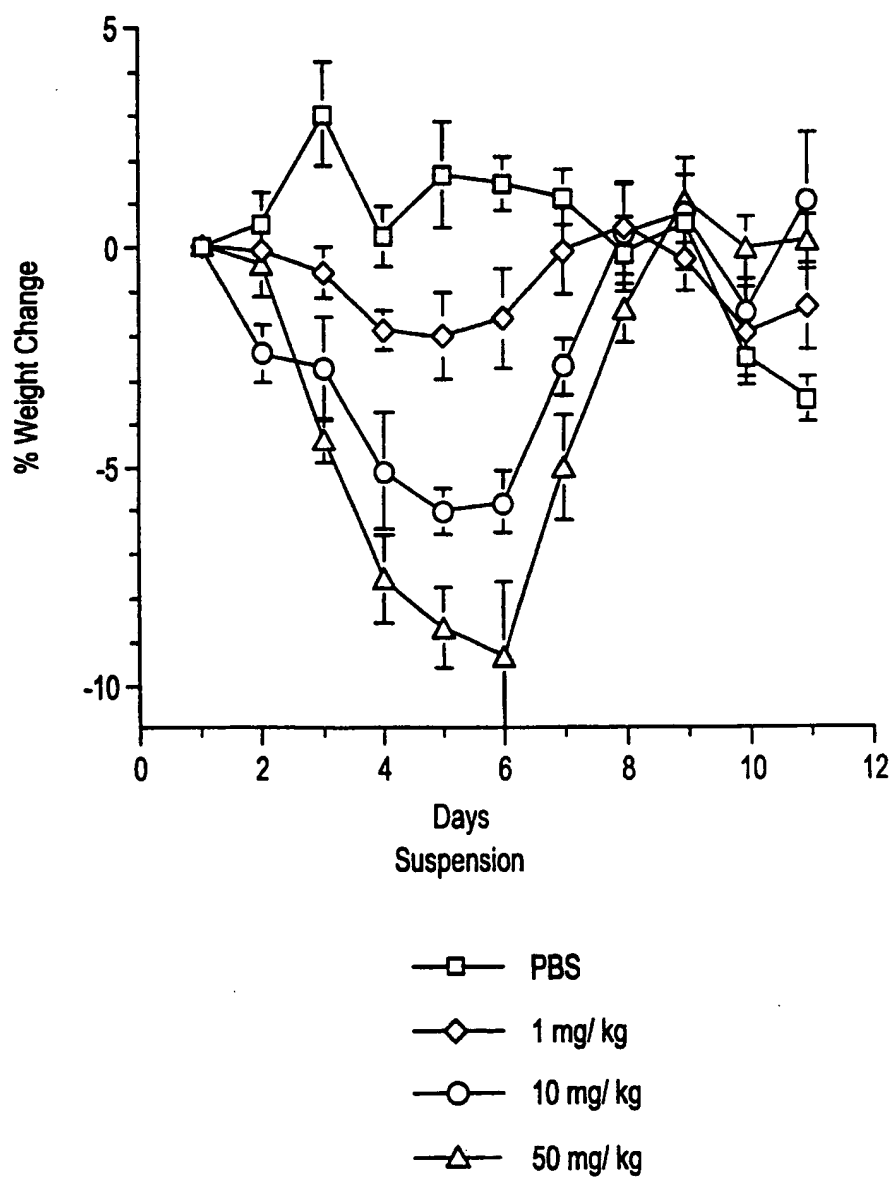
30 12. A method of claim 11 wherein said human
OB protein is resuspended in a diluent having a pH of
between 6.0 and 8.0.

- 29 -

13. A method of claim 11 wherein said human OB protein is rmetHu-Fc-leptin.

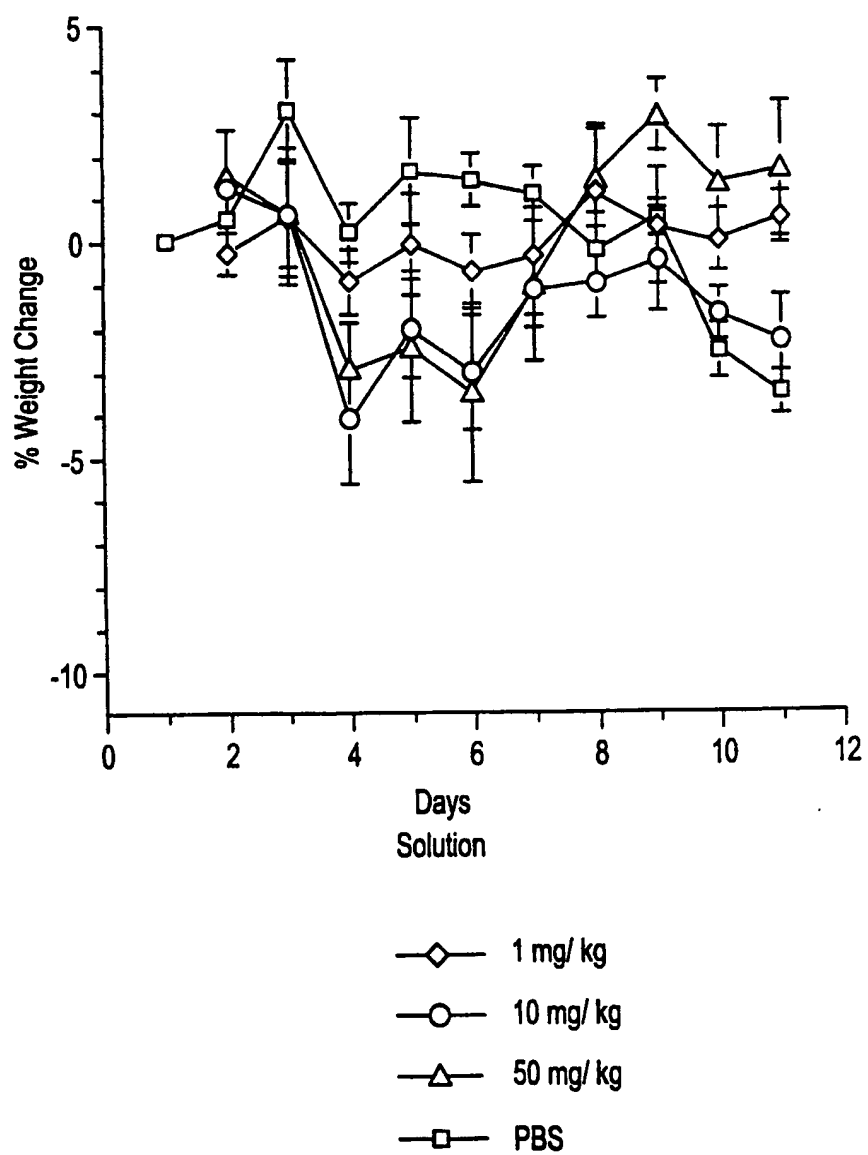
1 / 1 2

FIG. 1A



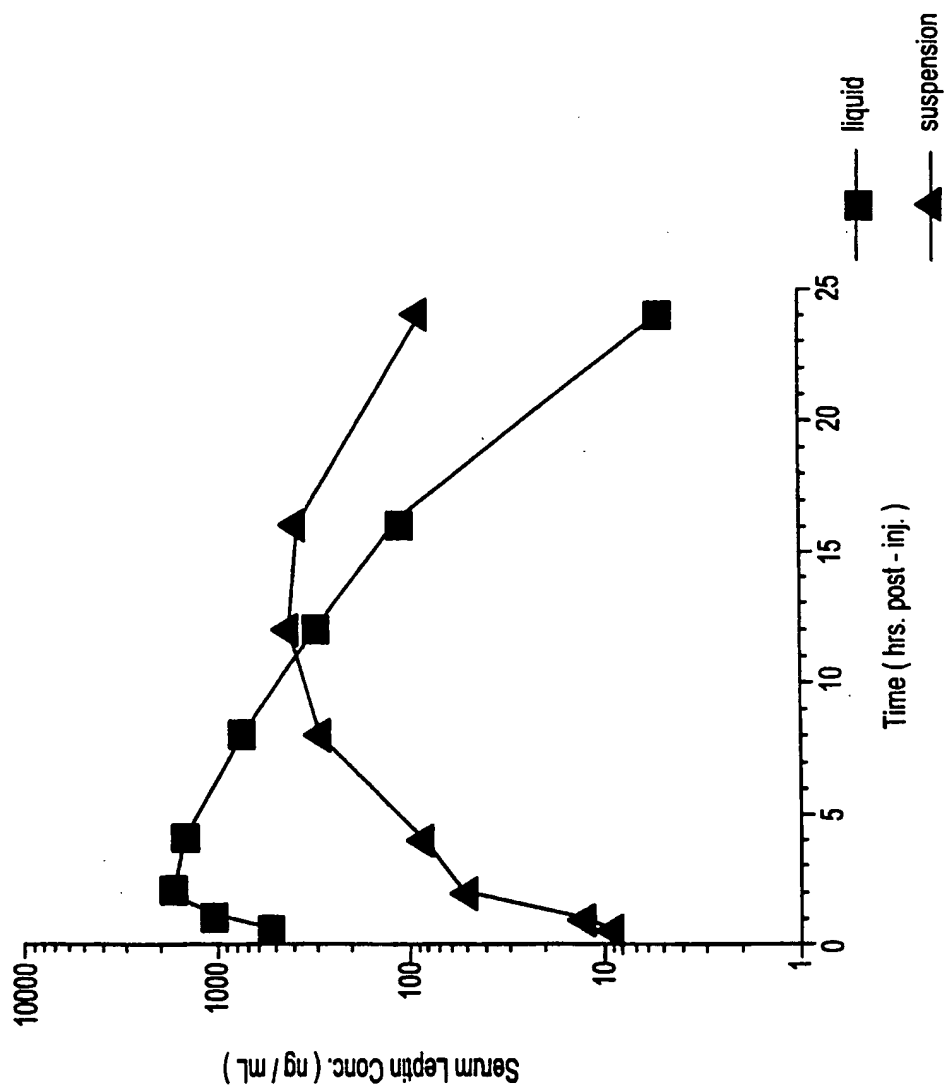
2 / 1 2

FIG. 1B



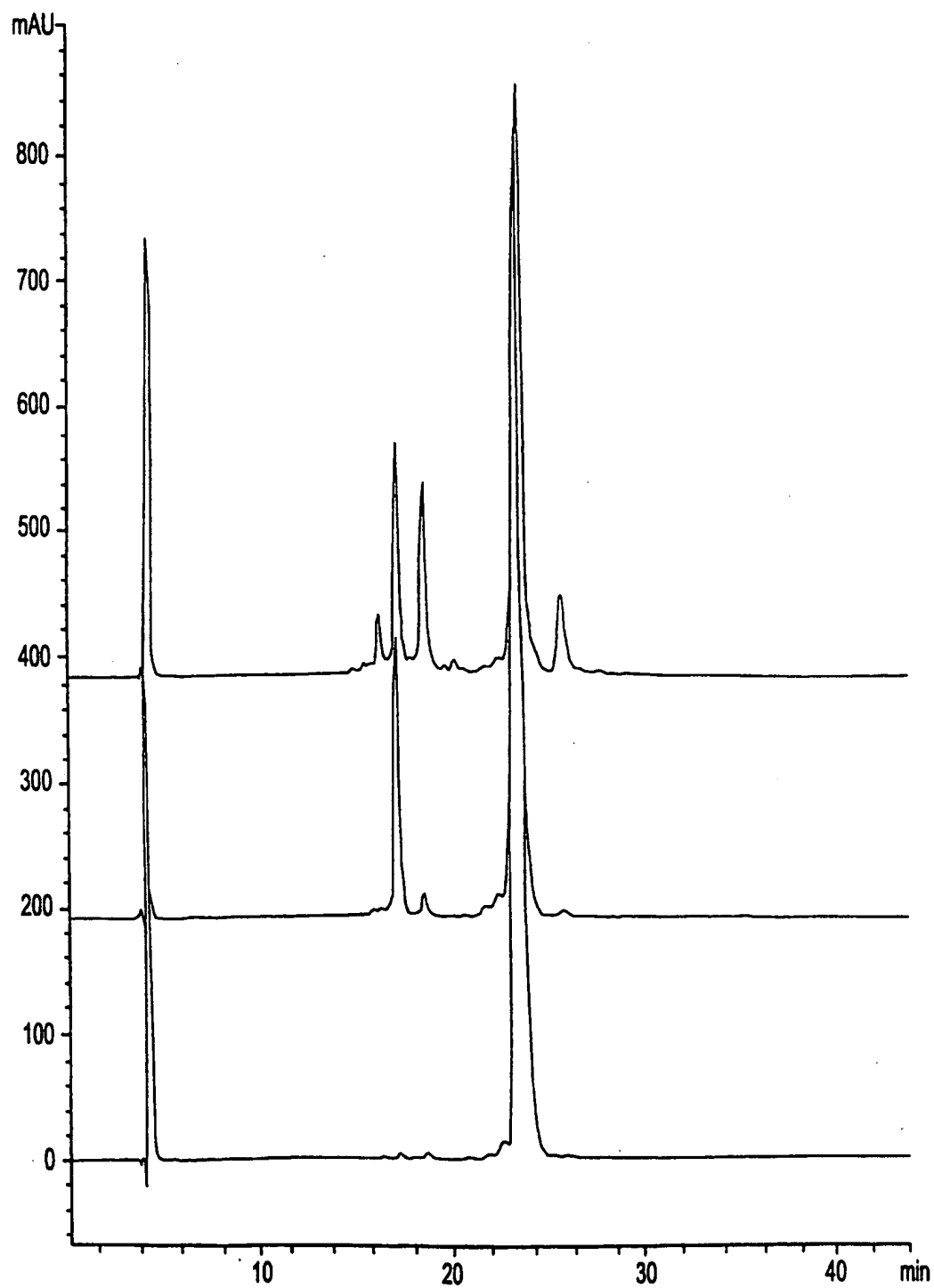
3 / 1 2

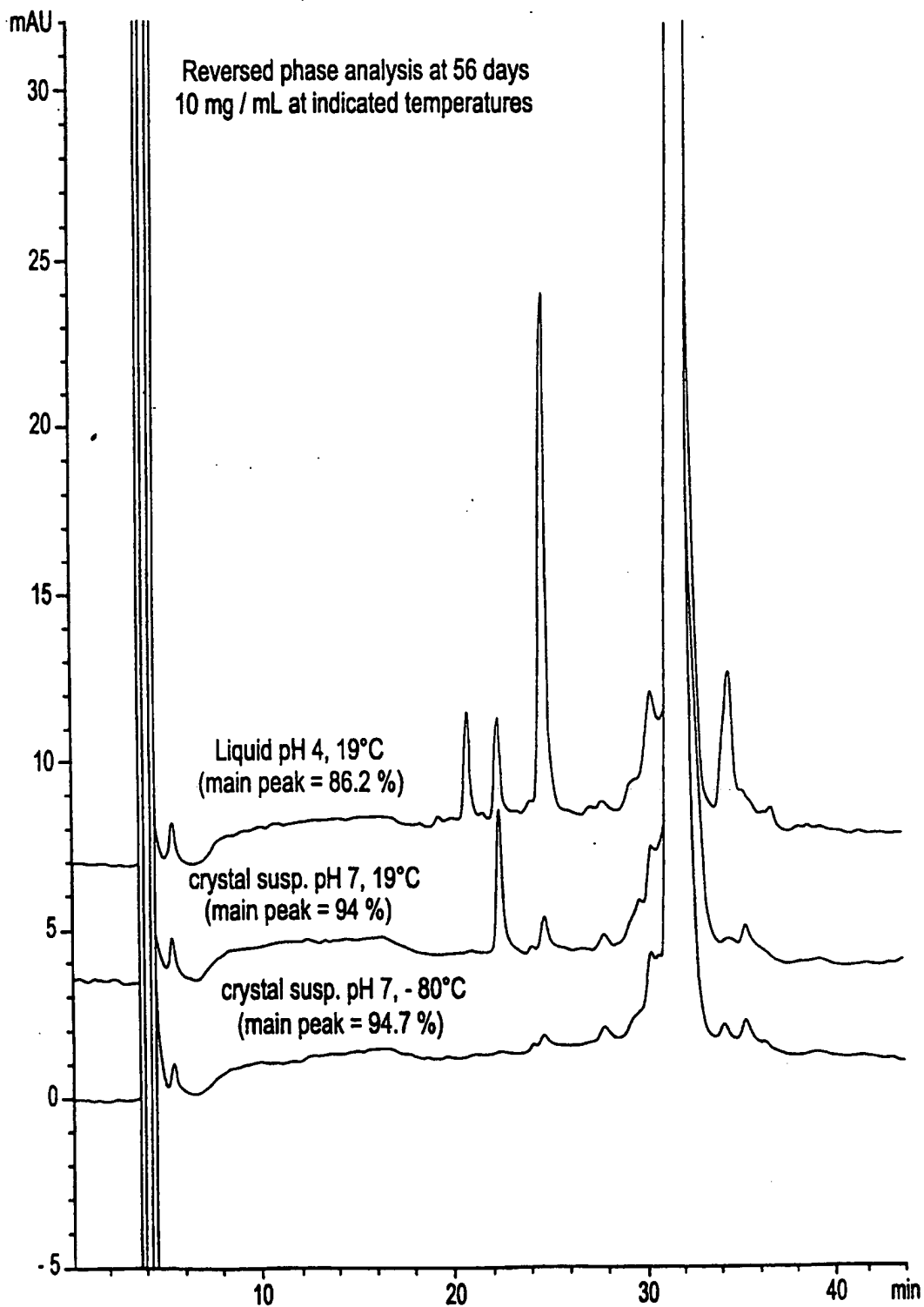
FIG.2



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FIG.3



5 / 1 2
FIG.4

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FIG.5A

Recombinant human metFc-OB (double stranded DNA) and
amino acid sequence (SEQ ID NOS: 1 and 2).

```
5      CATATGGAACCCAAATCTTGTGACAAACTCACACATGCCCACCGTGCCAGCACCTGAA
1  -----+-----+-----+-----+-----+-----+-----+-----+ 60
      GTATACCTTGGGTTTAGAACACTGTTTTGAGTGTGTACGGGTGGCACGGGTCGTGGACTT

10      M E P K S C D K T H T C P P C P A P E -
      CTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAACCCAAGGACACCCTCATGATC
61  -----+-----+-----+-----+-----+-----+-----+-----+ 120
      GAGGACCCCCCTGGCAGTCAGAAGGAGAAGGGGGTTTGGGTTCTGTGGGAGTACTAG

15      L L G G P S V F L F P P K P K D T L M I -
      TCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTC
121 -----+-----+-----+-----+-----+-----+-----+-----+ 180
20      AGGGCCTGGGGACTCCAGTGTACGCACCACCACCTGCACTCGGTGCTTCTGGGACTCCAG
      S R T P E V T C V V V D V S H E D P E V -

25      AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAG
181 -----+-----+-----+-----+-----+-----+-----+-----+ 240
      TTCAAGTTGACCATGCACCTGCCGCACCTCCACGTATTACGGTTCGTTCGGCGCCCTC

      K F N W Y V D G V E V H N A K T K P R E -

30      GAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCTCACCCTCCTGCACCAGGACTGG
241 -----+-----+-----+-----+-----+-----+-----+-----+ 300
      CTCGTCATGTTGTCGTGCATGGCACACCAGTCGCAGGAGTGGCAGGACGTGGTCTTGACC

      E Q Y N S T Y R V V S V L T V L H Q D W -

35      CTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAGCCCTCCAGCCCCCATCGAG
301 -----+-----+-----+-----+-----+-----+-----+-----+ 360
      GACTTACCGTTTCTCATGTTACGTTCCAGAGGTTGTTTCGGGAGGGTCGGGGGTAGCTC

40      L N G K E Y K C K V S N K A L P A P I E -
      AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCTGCCCCCA
361 -----+-----+-----+-----+-----+-----+-----+-----+ 420
      TTTTGGTAGAGGTTTCGGTTTCCCGTCGGGGCTCTTGGTGTCCACATGTGGGACGGGGGT

45      K T I S K A K G Q P R E P Q V Y T L P P -
      TCCCGGGATGAGCTGACCAAGAACCAGGTGACCTGACCTGCCTGGTCAAAGGCTTCTAT
421 -----+-----+-----+-----+-----+-----+-----+-----+ 480
50      AGGGCCCTACTCGACTGGTCTTGGTCCAGTCGGACTGGACGGACAGTTTCCGAAGATA

      S R D E L T K N Q V S L T C L V K G F Y -

55      CCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGAGAAACAATAAGACC
481 -----+-----+-----+-----+-----+-----+-----+-----+ 540
      GGGTCGCTGTAGCGGCACCTCACCTCTCGTTACCCGTCGGCCTCTTGTGATGTTCTGG

      P S D I A V E W E S N G Q P E N N Y K T -
```

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FIG.5B

5 541 ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGAC 600
-----+-----+-----+-----+-----+
TGCGGAGGGCACCACCTGAGGCTGCCGAGGAAGAAGGAGATGTCGTTTCGAGTGGCACCTG
T P P V L D S D G S F F L Y S K L T V D -

10 601 AAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCAC 660
-----+-----+-----+-----+-----+
TTCTCGTCCACCGTCGTCCCCTTGCAAGAGTACGAGGCACCTACGTACTCCGAGACGTG
K S R W Q Q G N V F S C S V M H E A L H -

15 661 AACCCTACACGAGAAGAGCCTCTCCCTGTCTCCGGGTAAAGTACCGATCCAGAAAGTT 720
-----+-----+-----+-----+-----+
TTGGTGATGTGCGTCTTCTCGGAGAGGGACAGAGGCCCATTTTCATGGCTAGGTCTTTCAA
N H Y T Q K S L S L S P G K V P I Q K V -

20 721 CAGGACGACACCAAAACCTTAATTAACGATCGTTACGCGTATCAACGACATCAGTCAC 780
-----+-----+-----+-----+-----+
GTCTGCTGTGGTTTTGGAATTAATTTGCTAGCAATGCGCATAGTTGCTGTAGTCAGTG
Q D D T K T L I K T I V T R I N D I S H -

25 781 ACCCAGTCGGTGAGCTCTAAACAGAAAGTTACAGGCCTGGACTTCATCCCGGGTCTGCAC 840
-----+-----+-----+-----+-----+
TGGGTCAGCCACTCGAGATTTGTCTTTCAATGTCCGGACCTGAAGTAGGGCCCAGACGTG
T Q S V S S K Q K V T G L D F I P G L H -

30 841 CCGATCCTGACCTTGTCAAAATGGACCAGACCCTGGCTGTATACCAGCAGATCTTAACC 900
-----+-----+-----+-----+-----+
GGCTAGGACTGGAACAGGTTTTACCTGGTCTGGGACCGACATATGGTCGTCTAGAATTGG
P I L T L S K M D Q T L A V Y Q Q I L T -

35 901 TCCATGCCGTCCCGTAACGTTATCCAGATCTCTAACGACCTCGAGAACCTTCGCGACCTG 960
-----+-----+-----+-----+-----+
AGGTACGGCAGGGCATTGCAATAGGTCTAGAGATTGCTGGAGCTCTTGAAGCGCTGGAC
S M P S R N V I Q I S N D L E N L R D L -

40 961 CTGCACGTGCTGGCATTCTCCAAATCCTGCCACCTGCCATGGGCTTCAGGTCTTGAGACT 1020
-----+-----+-----+-----+-----+
GACGTGCACGACCCTAAGAGGTTTAGGACGGTGGACGGTACCCGAAGTCCAGAACTCTGA
L H V L A F S K S C H L P W A S G L E T -

45 1021 CTGGACTCTCTGGGCGGGTCTGGAAGCATCCGGTTACAGCACCAGATTGTTGCTCTG 1080
-----+-----+-----+-----+-----+
GACCTGAGAGACCCGCCCCAGGACCTTCGTAGGCAATGTCGTGGCTTCAACAACGAGAC
L D S L G G V L E A S G Y S T E V V A L -

50 55

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FIG.5C

```
5      1081 TCCCGTCTGCAGGGTTCCTTCAGGACATGCTTTGGCAGCTGGACCTGTCTCCGGGTTGT
          -----+-----+-----+-----+-----+ 1140
          AGGGCAGACGTCCCAAGGGAAGTCCTGTACGAAACCGTCGACCTGGACAGAGGCCCAACA
          S R L Q G S L Q D M L W Q L D L S P G C -
10      TAATGGATCC
          1141 -----+ 1150
          ATTACCTAGG
          *
```

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FIG.6A

Recombinant human metFc-OB (double stranded) DNA and
amino acid sequence (SEO ID NOS: 3 and 4).

```
5      CATATGGACAAAACCTCACACATGTCCACCTTGTCAGCTCCGGAACCTCTGGGGGGTCTT
1      -----+-----+-----+-----+-----+-----+-----+ 60
      GTATACCTGTTTGTAGTGTGTACAGGTGGAACAGGTCGAGGCCTTGAGGACCCCCAGGA

10      M D K T H T C P P C P A P E L L G G P -

      TCAGTCTTCCTCTTCCCCCAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAG
61      -----+-----+-----+-----+-----+-----+-----+ 120
      AGTCAGAAGGAGAAGGGGGGTTTGGGTTCTGTGGGAGTACTAGAGGGCCTGGGGACTC

15      S V F L F P P K P K D T L M I S R T P E -

      GTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTAC
121      -----+-----+-----+-----+-----+-----+-----+ 180
      CAGTGTACGCACCACCACCTGCACTCGGTGCTTCTGGGACTCCAGTTCAAGTTGACCATG

20      V T C V V V D V S H E D P E V K F N W Y -

      GTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGC
25      181 -----+-----+-----+-----+-----+-----+-----+ 240
      CACCTGCCGCACCTCCACGTATTACGGTTCTGTTTCGGCGCCCTCCTCGTCATGTTGTGC

      V D G V E V H N A K T K P R E E Q Y N S -

30      ACGTACCGTGTGGTCAGCGTCCTCACCCTGCTGCACCAGGACTGGCTGAATGGCAAGGAG
241      -----+-----+-----+-----+-----+-----+-----+ 300
      TGCATGGCACACCAGTCGCGAGGAGTGGCAGGACGTGGTCTGACCGACTTACCGTTCCTC

35      T Y R V V S V L T V L H Q D W L N G K E -

      TACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAA
301      -----+-----+-----+-----+-----+-----+-----+ 360
      ATGTTACAGTTCAGAGGTTGTTTCGGGAGGGTCGGGGGTAGCTCTTTTGGTAGAGGTTT

40      Y K C K V S N K A L P A P I E K T I S K -

      GCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTG
361      -----+-----+-----+-----+-----+-----+-----+ 420
      CGGTTTCCCGTCGGGGCTCTTGGTGTCCACATGTGGGACGGGGTAGGGCCCTACTCGAC

45      A K G Q P R E P Q V Y T L P P S R D E L -

      ACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCC
421      -----+-----+-----+-----+-----+-----+-----+ 480
      TGGTTCTTGGTCCAGTCGGACTGGACGGACAGTTTCCGAAGATAGGGTCTGTAGCGG

50      T K N Q V S L T C L V K G F Y P S D I A -

      GTGGAGTGGGAGAGCAATGGGCAGCCGAGAACTACAAGACCACGCCTCCCGTGCTG
55      481 -----+-----+-----+-----+-----+-----+-----+ 540
      CACCTCACCTCTCGTTACCCGTCGGCCTCTGTTGATGTTCTGGTGGGAGGGGACGAC

      V E W E S N G Q P E N N Y K T T P P V L -
```


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FIG.6B

5 541 GACTCCGACGGCTCCTTCTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG 600
-----+-----+-----+-----+-----+
CTGAGGCTGCCGAGGAAGAAGGAGATGTCGTTTCGAGTGGCACCTGTTCTCGTCCACCGTC
D S D G S F F L Y S K L T V D K S R W Q -

10 601 CAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAG 660
-----+-----+-----+-----+-----+
GTCCCCTTGCAGAAGAGTACGAGGCACTACGTACTCCGAGACGTGTTGGTGATGTGCGTC
Q G N V F S C S V M H E A L H N H Y T Q -

15 661 AAGAGCCTCTCCCTGTCTCCGGGTAAAGTACCGATCCAGAAAGTTTCAGGACGACACCAAA 720
-----+-----+-----+-----+-----+
TTCTCGGAGAGGGACAGAGGCCCATTTTCATGGCTAGGTCTTTCAAGTCTGTGTTT
K S L S L S P G K V P I Q K V Q D D T K -

20 721 ACCTTAATTAACGATCGTTACGCGTATCAACGACATCAGTCACACCCAGTCGGTGAGC 780
-----+-----+-----+-----+-----+
TGGAATTAATTTTGCTAGCAATGCCATAGTTGCTGTAGTCAGTGTGGGTCAGCCACTCG
T L I K T I V T R I N D I S H T Q S V S -

25 781 TCTAAACAGAAAGTTACAGGCTGGACTTCATCCCGGTCTGCACCCGATCCTGACCTTG 840
-----+-----+-----+-----+-----+
AGATTGTCTTTCAATGTCCGACCTGAAGTAGGGCCAGACGTGGGCTAGGACTGGAAC
S K Q K V T G L D F I P G L H P I L T L -

30 841 TCCAAATGGACCAGACCCTGGCTGTATACCAGCAGATCTTAACCTCCATGCCGTCCCGT 900
-----+-----+-----+-----+-----+
AGGTTTACCTGGTCTGGGACCGACATATGGTCGTCTAGAATTGGAGGTACGGCAGGGCA
S K M D Q T L A V Y Q Q I L T S M P S R -

35 901 AACGTTATCCAGATCTCTAACGACCTCGAGAACCTTCGCGACCTGCTGCACGTGCTGGCA 960
-----+-----+-----+-----+-----+
TTGCAATAGGTCTAGAGATTGCTGGAGCTCTTGAAGCGCTGGACGACGTGCACGACCGT
N V I Q I S N D L E N L R D L L H V L A -

40 961 TTCTCCAAATCCTGCCACCTGCCATGGGCTTCAGGTCTTGAGACTCTGGACTCTCTGGGC 1020
-----+-----+-----+-----+-----+
AAGAGGTTTAGGACGGTGGACGGTACCCGAAGTCCAGAACTCTGAGACCTGAGAGACCCG
F S K S C H L P W A S G L E T L D S L G -

45 1021 GGGTCTCTGGAAGCATCCGGTTACAGCACCGAAGTTGTTGCTCTGTCCCGTCTGCAGGGT 1080
-----+-----+-----+-----+-----+
CCCCAGGACCTTCGTAGGCCAATGTCGTGGCTTCAACAACGAGACAGGGCAGACGTCCCA
G V L E A S G Y S T E V V A L S R L Q G -

50 55

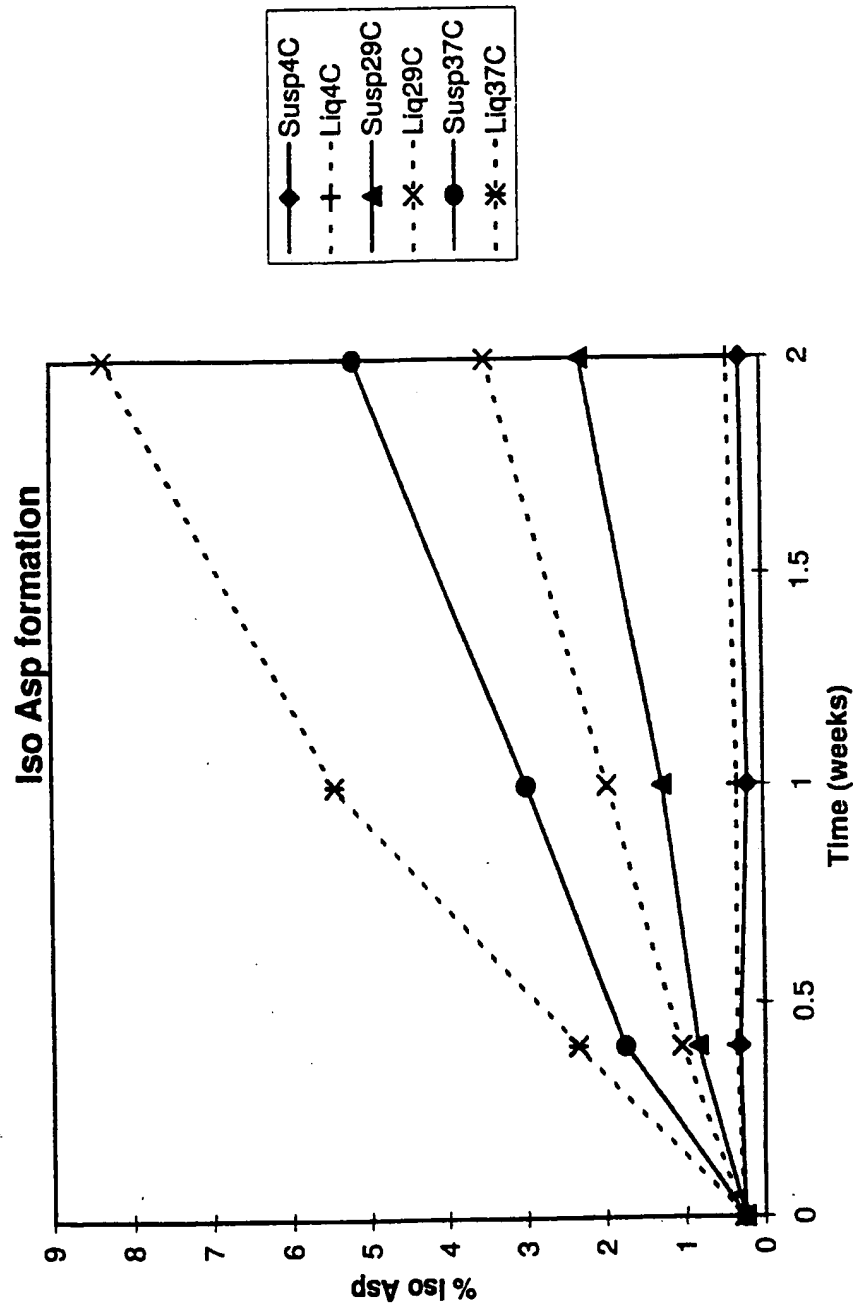
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FIG.6C

5 1081 TCCCTTCAGGACATGCTTTGGCAGCTGGACCTGTCTCCGGGTTGTTAATGGATCC 1135
 -----+-----+-----+-----+-----
 AGGGAAGTCCTGTACGAAACCGTCGACCTGGACAGAGGCCCAACAATTACCTAGG
 S L Q D M L W Q L D L S P G C * -
10

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FIG.7



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/07828

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 A61K38/22		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 A61K C07K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 00319 A (SMITHKLINE BEECHAM PLC ;BROWNE MICHAEL JOSEPH (GB); CHAPMAN CONRAD) 3 January 1997 see page 7, line 33 - line 35 see page 8, line 16 - line 22; claims; examples	1-13
P, X	WO 97 24440 A (GENENTECH INC ;SAUVAGE FREDERIC J DE (US); LEVIN NANCY (US); VANDL) 10 July 1997 see claims; examples	10-13
A	WO 96 23518 A (LILLY CO ELI ;BASINSKI MARGARET B (US); DIMARCHI RICHARD D (US); H) 8 August 1996 see page 25, line 10 - line 19 --- -/--	1-9
<div style="display: flex; justify-content: space-between;"> <input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex. </div>		
<div style="display: flex;"> <div style="flex: 1;"> <p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="flex: 1;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"Z" document member of the same patent family</p> </div> </div>		
Date of the actual completion of the international search <div style="text-align: center; font-weight: bold;">27 August 1998</div>		Date of mailing of the international search report <div style="text-align: center; font-weight: bold;">03/09/1998</div>
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3018		Authorized officer <div style="text-align: center; font-weight: bold;">Fuhr, C</div>

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/07828

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	<p>WO 98 28427 A (AMGEN INC) 2 July 1998 see claims; examples -----</p>	10-13

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/07828

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 10-13
are directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/07828

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9700319 A	03-01-1997	AU 6011096 A EP 0832219 A	15-01-1997 01-04-1998
WO 9724440 A	10-07-1997	AU 1520097 A	28-07-1997
WO 9623518 A	08-08-1996	US 5559208 A AU 4862096 A CA 2211801 A EP 0809508 A	24-09-1996 21-08-1996 08-08-1996 03-12-1997
WO 9828427 A	02-07-1998	NONE	